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GASTROINTESTINAL PARASITES OF THE SAIMAA RINGED SEAL (*PUSA HISPIDA SAIMENSIS*)

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The aim of this study was to examine the distribution of parasites in the intestinal tract of the Saimaa ringed seal (*Pusa hispida saimensis*). The material used in this study was collected from seal individuals which were found dead in Lake Saimaa between years 2004–2012. The age of the individuals varied from zero to five years, of which six were pups less than one year old.

The examinations were performed in the facilities at the University of Eastern Finland in Joensuu in May 2014. The intestines were separated from the stomach, and the stomach was weighed. The different parts of the intestinal tracts were then separated from each other. The large and small intestines were measured; the large intestine was cut into two, and the small intestine into ten, equally long pieces. After this procedure, the intestines were opened using blunt-ended scissors and the insides were examined. Parasites were collected with a pair of tweezers and preserved in 99.5% ethanol, each placed into a Sarstedt tube labelled according to the specimen and the part of the tract where it was recovered from. The otholites were placed on a petri dish that was labelled according to the specimen and whether the samples were from stomach or intestines. All in all nine seal intestines were examined, and the total amount of parasites gathered was 1,429.

Although the sampling of this work remained rather small, the results support the previous findings in many ways. Out of the nine examined samples, parasites were found from six of them. There were no parasites in three of the youngest pups. The parasites found were exclusively gastrointestinal worms known as *Corynosoma magdaleni.* This study shows that these worms are clearly adapted to living in the middle section of the small intestine. No worms were found from the large intestine, cecum, or from the first section of the small intestine.

Saimaa ringed seal is a critically endangered species. In view of conservation, every study performed on this seal species is extremely important. The study of the seal parasites helps determine the general state of health of this animal, and plan further the protection of the species.

KEYWORDS:

Saimaa ringed seal, seal, parasite, gastrointestinal, Acanthocephala, Corynosoma magdaleni

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SAIMAANNORPAN (PUSA HISPIDA SAIMENSIS) SUOLISTOLOISET

Tämän tutkimuksen tarkoituksena oli kartoittaa saimaannorpan (*Pusa hispida saimensis*) suolistoloisten levinneisyyttä suoliston eri osissa. Tutkimuksessa käytetty materiaali oli kerätty Saimaan vesistöstä kuolleina löydetyistä norppayksilöistä vuosina 2004–2012. Yksilöiden ikä vaihteli välillä 0–5 vuotta. Kuusi yhdeksästä yksilöstä olivat alle vuoden ikäisiä poikasia.

Suolten avaukset tehtiin Itä-Suomen yliopiston tiloissa Joensuussa vuoden 2014 toukokuussa. Suolet leikattiin erilleen mahasta, ja maha punnittiin. Suolen eri osat leikattiin toisistaan erilleen. Paksusuoli ja ohutsuoli mitattiin, minkä jälkeen paksusuoli leikattiin kahteen ja ohutsuoli kymmeneen yhtä suureen osaan. Tämän jälkeen suolet avattiin suolisaksilla ja sisäosat käytiin silmämääräisesti läpi. Loiset kerättiin suolen seinämästä pinseteillä ja säilöttiin 99,5-prosenttista etanolia sisältäviin Sarstedt-putkiin sen mukaan, mistä yksilöstä ja suolen osasta näyte oli kerätty. Otoliitit sekä muut kalan osat asetettiin merkatulle petrimaljalle kuivumaan. Kaiken kaikkiaan yhdeksän norppayksilön suolet avattiin, joista loisia kerättiin yhteensä 1 429 kappaletta.

Tämän työn otoskoko jäi pieneksi, mutta tulokset tukevat aikaisempia tutkimuksia monin tavoin. Yhdeksästä avatusta suolesta yhteensä kuudesta löytyi loisia. Kolmesta nuorimmasta poikasesta ei löytynyt loisia. Löydetyt loiset olivat yksinomaan *Corynosoma magdaleni* -väkäkärsämatoja. Madot ovat selvästi erikoistuneet elämään ohutsuolen keskivaiheilla, avauksissa ei yhdestäkään näytteestä löytynyt loisia paksu- ja umpisuolesta eikä ohutsuolen alkupäästä.

Saimaannorppa on erittäin uhanalainen laji. Tätä näkemystä vasten on jokainen norpalle tehty tutkimus tärkeä luonnonsuojelun näkökulmasta. Norpan loisten kartoitus auttaa selvittämään tämän nisäkäsalalajin terveydellistä tilaa, jonka pohjalta voidaan laatia suunnitelmia lajin suojelun edistämiseksi.

ASIASANAT:

Saimaannorppa, norppa, loinen, suolisto, Acanthocephala, Corynosoma magdaleni

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1 INTRODUCTION

In previous centuries, local people considered the seals to be competitors with fisheries, and currently skeptical attitudes have been expressed towards the extension of fishing restrictions related to seal conservation in Lake Saimaa (Auttila et al, 2014). Although the ringed seal diet in the marine environment has been well studied, only a few studies concerning the diet and foraging behavior of freshwater ringed seals have been conducted so far (Kunnasranta et al. 1999, Sinisalo 2007). The most common method of studying the diet composition in pinnipeds is the identification of prey remains from faeces or within the digestive tracts (Sinisalo 2007).

Studies of the intestinal helminth (parasitic worm) parasites of seals offer an alternative approach to study the foraging behaviour of individuals (Sinisalo, 2007). On average, a seal's digestive tract can be empty within 6–8 hours of feeding (Parsons, 1977), so it is extremely difficult to build a reliable picture of the species' diet in the long run without a large sample size. So, especially Acanthocephalan (spiny-headed worms) species are good indicators of the past diet over a long term, as they can live within the host for weeks (Valtonen & Helle, 1982).

The Saimaa ringed seal, *Pusa hispida saimensis*, inhabits the lake Saimaa in eastern Finland. Amounting to circa 310 individuals, with an annual birth rate of some 50–60 pups, this subspecies is currently listed as critically endangered in the Red List of Finnish Species (Rassi et al. 2010).

Studies of Saimaa ringed seal's parasites have previously been conducted in the years 1981–2001. These studies showed that contagions are relatively rare in pups under the age of two months, but rapidly increase to almost 40% within fish-eating few-month-olds. By the time the seals reach the age of 18 months, over 80 % of seals contain parasites, with the average parasite number of 60. The older the seals get, the more common gastrointestinal parasite infections

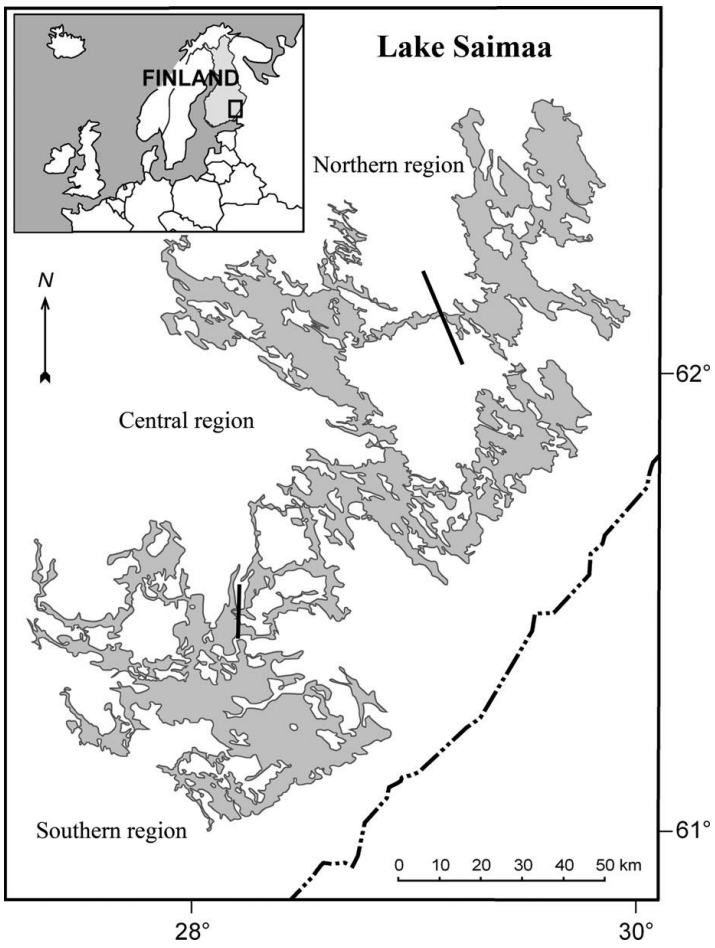
become. Before the current thesis, the largest found population of *Corynosoma magdaleni* in one seal individual was 347 (Valtonen et al, 2012).

The aim of this study was to examine the distribution of Corynosoma magdaleni in the intestinal tract, and to support the previously conducted studies.

2 BIOLOGY

2.1 The Saimaa ringed seal *(Pusa hispida saimensis)*

The Saimaa ringed seal is a small, landlocked subspecies that lives exclusively in Lake Saimaa in eastern Finland (Picture 1). The differentiation of this subspecies began during the last ice age 11 000 years ago when the Baltic ringed seal *(Pusa hispida botnica)* was cut off from the stocks of the Arctic ringed seal *(P. h. hispida)* (Sinisalo et al, 2003). Since then, the Saimaa ringed seal and also its parasites have lived geographically isolated in a freshwater environment (Valtonen & Helle, 1988; Hyvärinen & Nieminen, 1990)



Picture 1 Lake Saimaa, Finland (Auttila et al, 2014).

Ice and snow form a vital nesting site for the seals. Saimaa seals give birth around February–March (Ympäristöministeriö, 2011) in lairs excavated in snowdrifts along island shores. The pup suckles for seven to nine weeks, after which the average weight of a pup is approximately 20 kg, it stays within the proximity of the nest for as long as there is some nest structures left (Rautio et al, 2009, Niemi et al. 2011).

The diet of this seal subspecies consists mainly of small (average size 10cm) shoaling fish species such as perch (*Perca fluviatilis*), common roach (*Rutilus rutilus),* vendace (*Corgenous albula*), smelt (*Osmerus eperlanus*) and ruffe (*Gymnocephalus cernuus*) (Kunnasranta et al, 1999; Sinisalo, 2007; Ympäristöministeriö, 2011).

Seals eat on average two kilograms of fish a day. In autumn, they start to build their fat layer (also known as blubber) for heat isolation. During that time they can eat up to four kilos of fish per day. Annually the amount of fish consumed by one Saimaa ringed seal is on average a thousand kilograms (Kunnasranta et al, 1999).

Saimaa ringed seals can live up to and over 30 years of age, with an average life expectancy of 20 years (Hyvärinen et al, 2004). This seal subspecies is the only endemic species of fauna found in Finland. The current population is estimated to be 310 individuals (Ympäristöministeriö, 2011).

2.2 Gastrointestinal parasites of seals

A rich parasite fauna can be found in marine mammals worldwide. Gastrointestinal acanthocephalans (genus *Bolbosoma* and *Corynosoma*) are found in ocean cetaceans as well as pinnipeds worldwide (Dailey, 2005). In seals around the Baltic, a total of 62 different helminth species have been found, of which ten are acanthocephalans (Dailey, 1975; Sinisalo, 2007).

Corynosoma species are polygamous parasitic worms that mature and grow in the intestinal tract of seals (Dailey 2005). They copulate in the tract of their main

host, producing acanthocephalan eggs (acanthor). The acanthors then leave the main host either alongside faeces or with their dead mother, into the water. The *Corynosoma* only have one intermediate host, in this case the *Monoporeia affinis* (a species of amphipod), where the next stage, the cystacanth larvae, is developed. The amphipod is eaten by a fish, which now works as a paratenic (transfer) host. The parasite larvae encapsulate within the paratenic host, and can stay infectious for years. When the fish is eaten by a seal, the parasites activate in the intestinal tract and mature (Valtonen et al, 2012; Sinisalo, 2007).

In Saimaa ringed seals, only one species of gastrointestinal parasite has been found: *Corynosoma magdaleni*. Two other species of *Corynosoma*, which dwell in the Baltic Sea, have either not been able to handle the freshwater conditions, or the seals that originally founded the Saimaa population were not infected by them. The life cycle of *C. magdaleni* has been enabled by the presence of *Monoporeia affinis*, their intermediate host, in Saimaa (Valtonen et al. 2012).

3 MATERIALS AND METHODS

3.1 Samples

The bodies of the dead Saimaa ringed seals used in this study were collected from Lake Saimaa between years 2004–2012. All sample specimens were found dead, and had either died of natural causes or by incidental by-catch. A total of nine intestines were examined, of which six contained gastrointestinal parasites.

The age of the seals varied from zero to five years (zero being a pup). Five of them were females, and four males. Their weight distribution was between 4.3kg and 53 kg, and their body lengths varied from 69 cm to 122 cm.

The sections of the intestinal tracts were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

3.2 The examination of the intestines

The samples were analysed in Joensuu, in a preparation laboratory at the University of Eastern Finland between May 13 and May 16 2014.

The large and small intestines of the seal specimens had previously been removed from the bodies, and stored in a freezer at a mean temperature of -20°C. Before the analysis could be conducted, the samples were removed from the freezer, and left to thaw at room temperature for 24 hours.

At the beginning of the preparation work, the intestines were first separated from the stomach. The stomach was weighed both when both full and empty (Picture 2).

Picture 2 Stomach of Saimaa ringed seal, separated from the intestines.

The intestines were then laid out on the preparation table and measured. To straighten them out, the loops were first cut off from the mesentery (a thin layer of tissue keeping the intestines together) using a pair of small scissors. After the measurements, the intestines were cut into three parts; the small intestine, the large intestine and the cecum (Picture 3). Furthermore, the small intestine was cut into ten equally sized parts, and the large intestine was cut into half, to enable a more precise inspection of the inside of the intestine, and the location of parasites.

Picture 3 The intestines of a seal specimen, with different parts (cecum, large intestine and small intestine) separated for further analysis.

The pieces of the intestine were then examined individually. This procedure began by cutting the part of the intestine open lengthwise with a pair of blunt-ended scissors. Then the piece was pulled open using small tweezers, and a small amount of water was used to carefully remove any excess blood or other intestinal contents (Picture 4). The inner surface of the sample was then examined for parasites and otholites (Picture 5).

Picture 4 A piece of the small intestine ready to be examined for parasites.

All worms were collected with a pair of tweezers and preserved in 99.5% ethanol, each placed into a Sarstedt tube labelled according to the specimen and the part of the tract where it was recovered from. The otholites were placed on a petridish that was labelled according to the specimen and whether the samples were from the stomach or intestines.

Picture 5 *Corynosoma* worms attached to the inner wall of the intestine (Amin et al, 2011).

The analysis was complicated by the different stages of decay in which the samples were. If the specimen is not fresh, the intestines are very flaccid and discoloured, or inflated with gas (Fay et al, 1979). Also, the parasites were often discoloured, and in some cases, too decomposed for further examination.

4 RESULTS

Among the nine specimens in this study, gastrointestinal parasites were found from six of them. For a clearer textual context, the samples were named as specimens A, B, C, D, E and F. Figures 1-6 demonstrate the location of the worms found within the digestive tract of each seal specimen, and also show the number of them found in each section of the intestines.

4.1 Specimen A

Specimen A was a female pup, which was found dead in June 2011. The cause of death for this individual is unknown. It weighed 17.5 kg and was 84 cm long. The length of the small intestine was 947 cm, and that of the large intestine was 34.5 cm. The total number of parasites found from this specimen was 165. There were no parasites in the small intestines sections 1, 2 and 10, nor were there any in the large intestine or cecum. By far the highest density of *C. magdaleni* was found in section 7 (Figure 1).

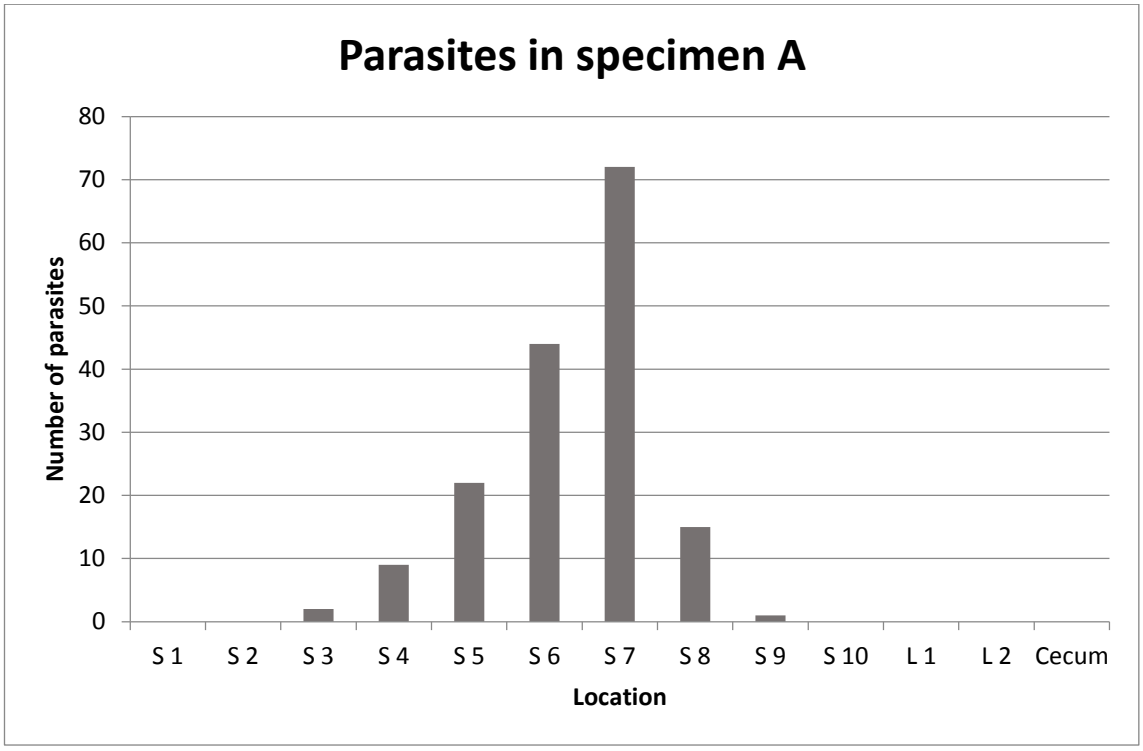


Figure 1 The parasite distribution in the guts of specimen A. The sections were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

4.2 Specimen B

Specimen B was a three-year-old male which had died in 2011. This seal had died of blood loss caused by deep lacerations. Injuries were suspected to have come from a propeller of a boat, but cannot be confirmed. The seal weighed 53 kg and was 122 cm long. Its small intestine was 940 cm long, and the large intestine being 47.5 cm. The number of parasites found in this individual was 8. No worms were found in sections 1, 4, 8–10, or from the large intestine or cecum. The highest density of *C. magdaleni* was found in section 6 (Figure 2).

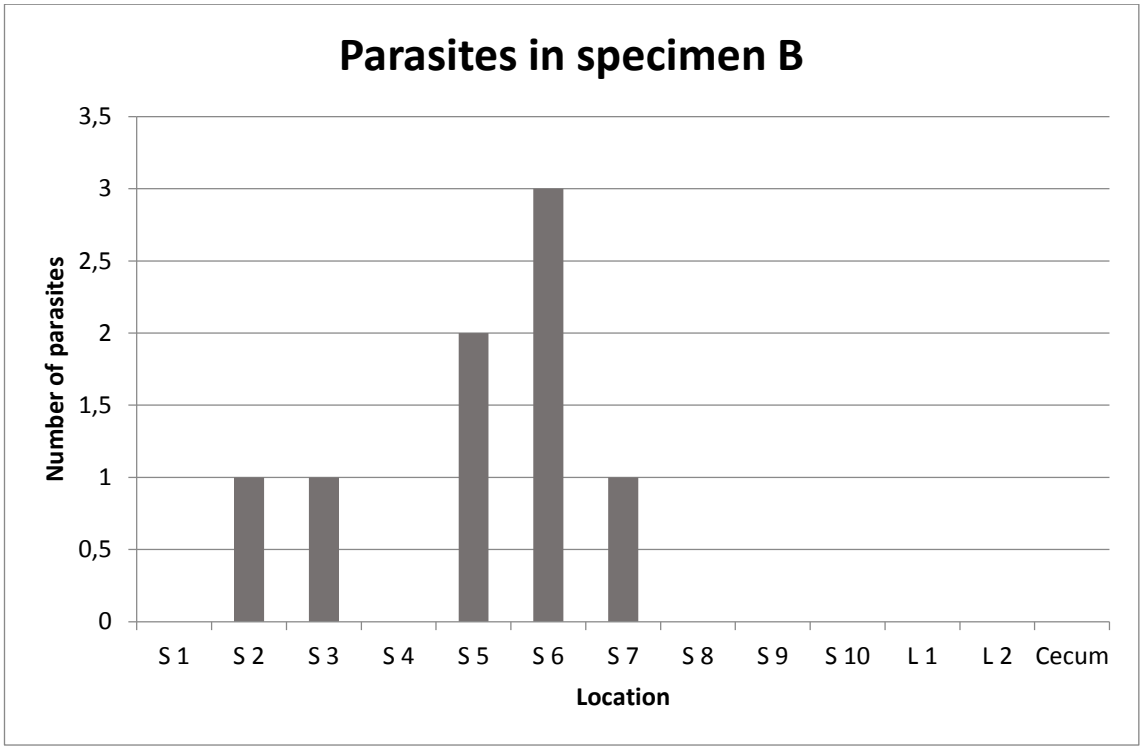


Figure 2 The parasite distribution in the guts of specimen B. The sections were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

4.3 Specimen C

Specimen C was a one-year-old female. The time of death was estimated to have been somewhere within year 2012. This individual's body showed signs of asphyxiation, but this cannot be verified due to the high level of decay of the corpse. The female weighed 41kg and was 103 cm long. Its small intestine was 976 cm in length, and the large intestine was 22 cm. The total number of parasites found from this specimen was 1 094, which is by far the largest number ever discovered in a Saimaa ringed seal. The intestinal tract was completely empty of any fecal matter or food, and seemed to be highly infected, the walls of the intestines being very dark red in color. No parasites were found from section 1 of the small intestine, or from the large intestine or cecum. The highest density of worms was found in sections 6–8 of the small intestine (Figure 3).

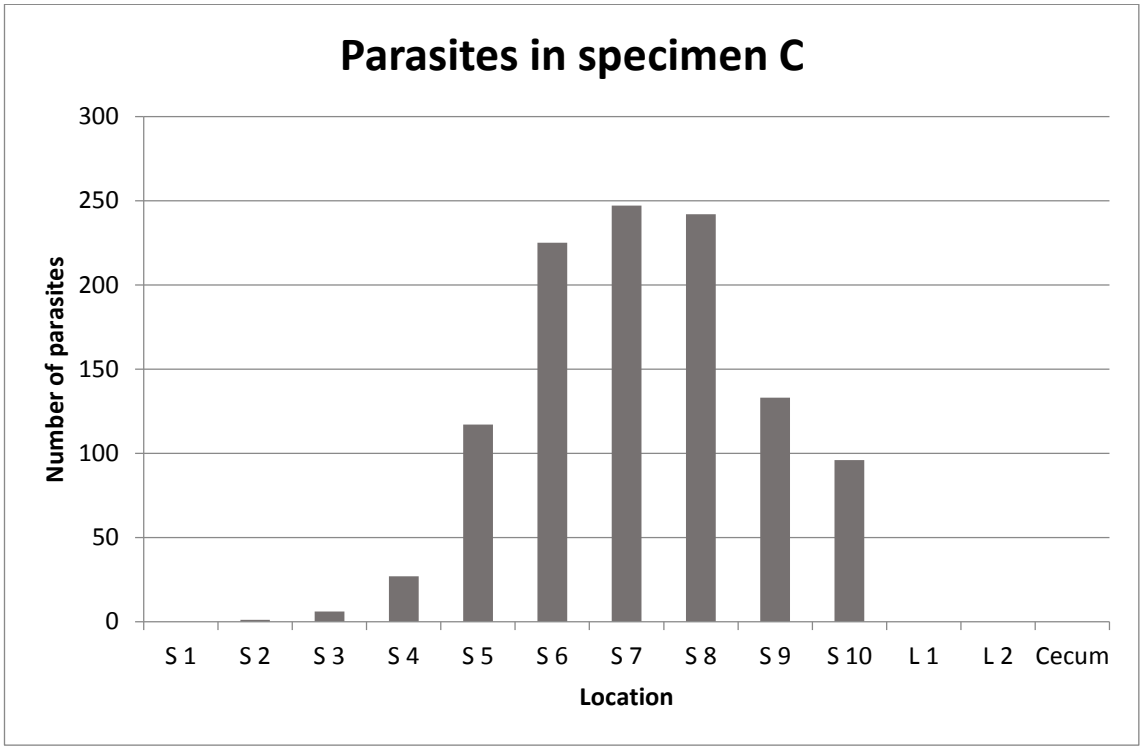


Figure 3 The parasite distribution in the guts of specimen C. The sections were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

4.4 Specimen D

In this specimen the large intestine and cecum had previously been removed, and were not recovered. No data was found on them and therefore this sample would not qualify for official comparisons.

This seal was a male, five years of age, which had died in June of 2009. The body had drifted to shore, and the seal had died while its founders had reported it to the authorities. It suffered from erysipeloid (a zoonotic bacterial skin infection), a broken phalanx and deep, infected lacerations. Its weight was measured as 32.5 kg, and it was 113 cm in length. The small intestine was 973 cm long. This specimen had 115 parasites in total. The worms were scattered throughout the small intestine apart from the first section. The highest density of them was found in section 6 of the small intestine (Figure 4).

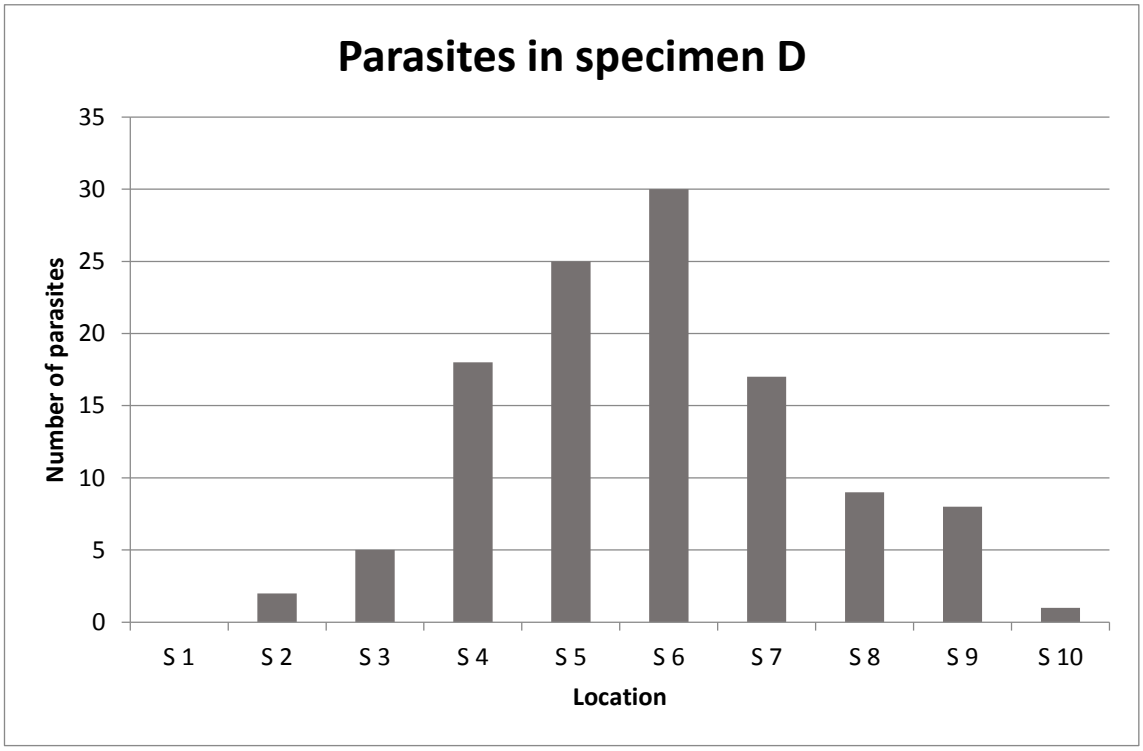


Figure 4 The parasite distribution in the guts of specimen D. The sections were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

4.5 Specimen E

Specimen E was a pup of female gender. The time of death was estimated to be somewhere around year 2011. The body showed signs of drowning, but the cause of death could not be confirmed due to the severe decay the body was in. This pup weighed 12 kg and was 91 cm long. The length of the small intestine was 500 cm, and that of the large intestine 33.5 cm. The total number of parasites found in the body was 42 (Figure 5). Unlike in the other specimens in this study, the highest parasite density in this individual was in the fourth section of the small intestine, closer to the stomach than in the other examined individuals. No worms were recovered from the first, eighth, ninth and tenth sections of the small intestine, the large intestine, and the cecum.

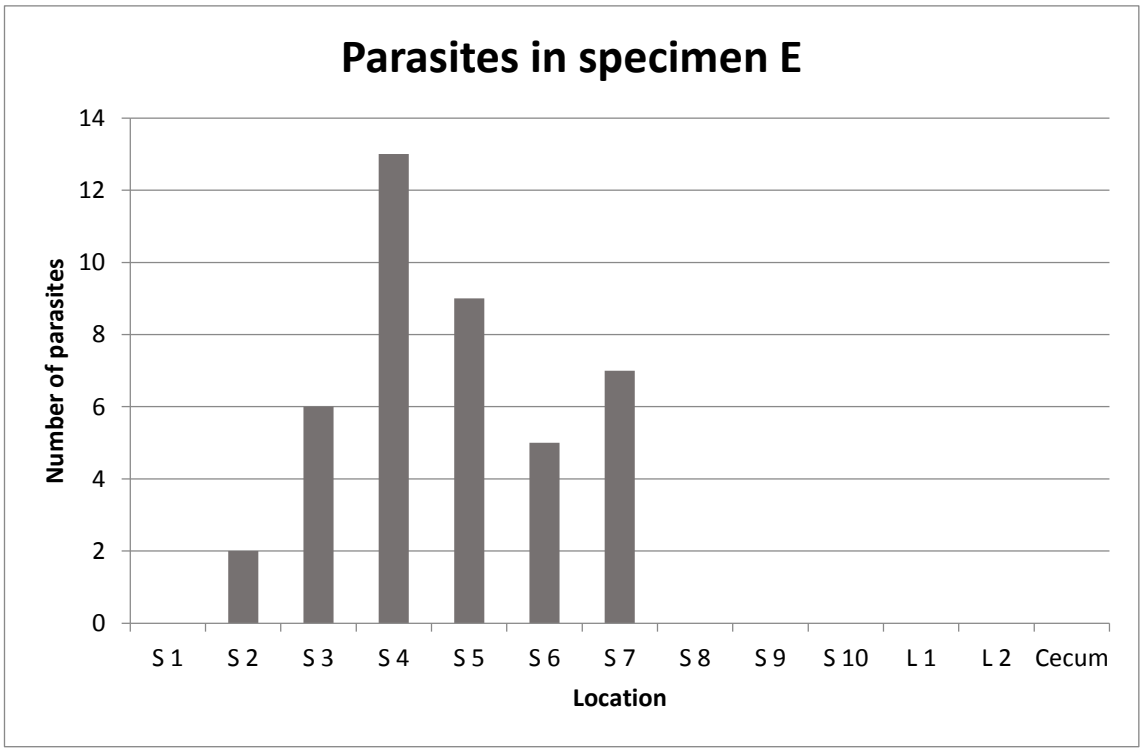


Figure 5 The parasite distribution in the guts of specimen E. The sections were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

4.6 Specimen F

Specimen F was a male pup. The time of death was estimated to have been within year 2011 as a by-catch. The weight of this individual was 21 kilograms, and it was 91 centimeters in length. The small intestine was 889 centimeters long, and the large intestine length was 30 cm. Parasites were found from sections four to six in the small intestine. The total number of parasites in this individual was five (Figure 6).

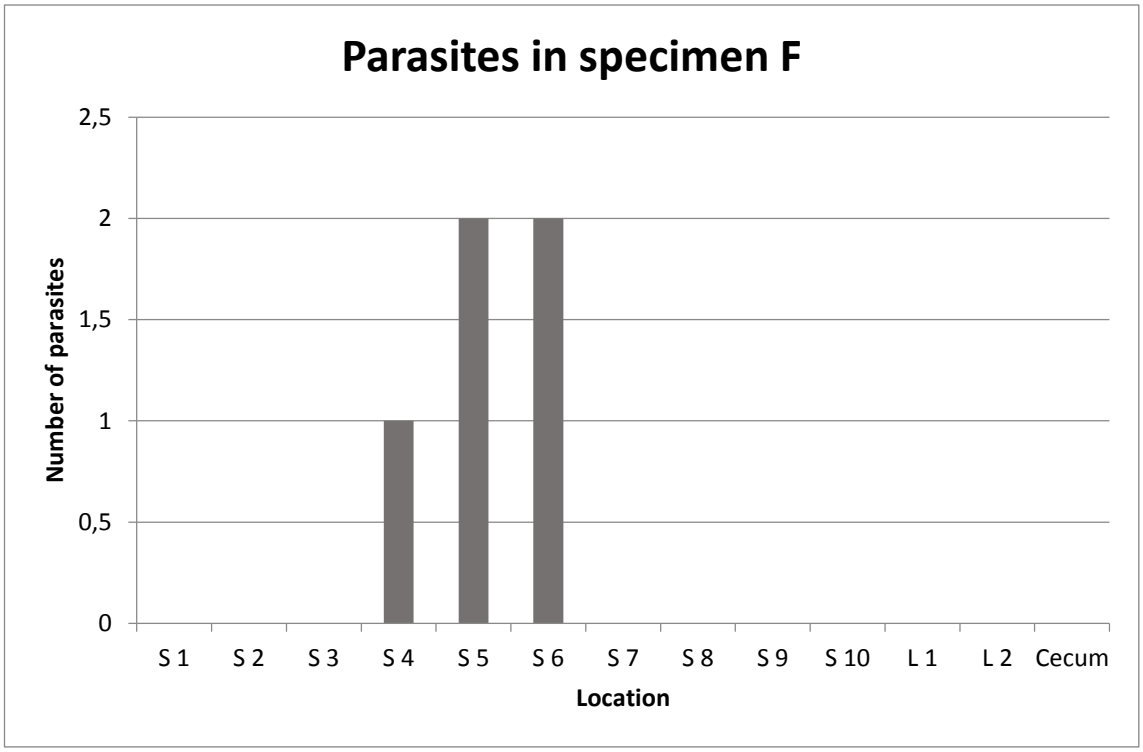


Figure 6 The parasite distribution in the guts of specimen F. The sections were named as S 1–10 for the sections of the small intestine, and L1 and L2 for the sections of the large intestine.

5 DISCUSSION

5.1 Site segregation

In the studies performed by Sinisalo et al in 2004, *Corynosoma magdaleni* was always found along the full length of the small intestine, but never in the large intestine, cecum or rectum. In the analyses conducted in the current study, parasites were never found in the first section of the small intestine, large intestine or in the cecum. The analysis of the species of parasites found in this study was not conducted, because an earlier study had proven that only one species exists in Saimaa ringed seal.

In the Baltic Sea, *Corynosoma* species are separated in the intestinal tracts of grey seals (*Halichoerus grypus*) and ringed seals. The majority of *C. semerme* specimens are located in the large intestine and in the rectum (Valtonen & Helle, 1988; Nickol, Valtonen & Helle, 2002). The preferred sites of *Corynosoma magdaleni* in Lake Saimaa seals in the current study as well as according to Sinisalo et al. 2014 were in the middle sections of the small intestines (Figures 7 & 8). This indicates an adaptation, perhaps influenced by competition with other *Corynosoma* species in the past, which lead to a selective segregation that has been maintained after the Saimaa seals were isolated from the Baltic Sea population (Sinisalo et al, 2004).

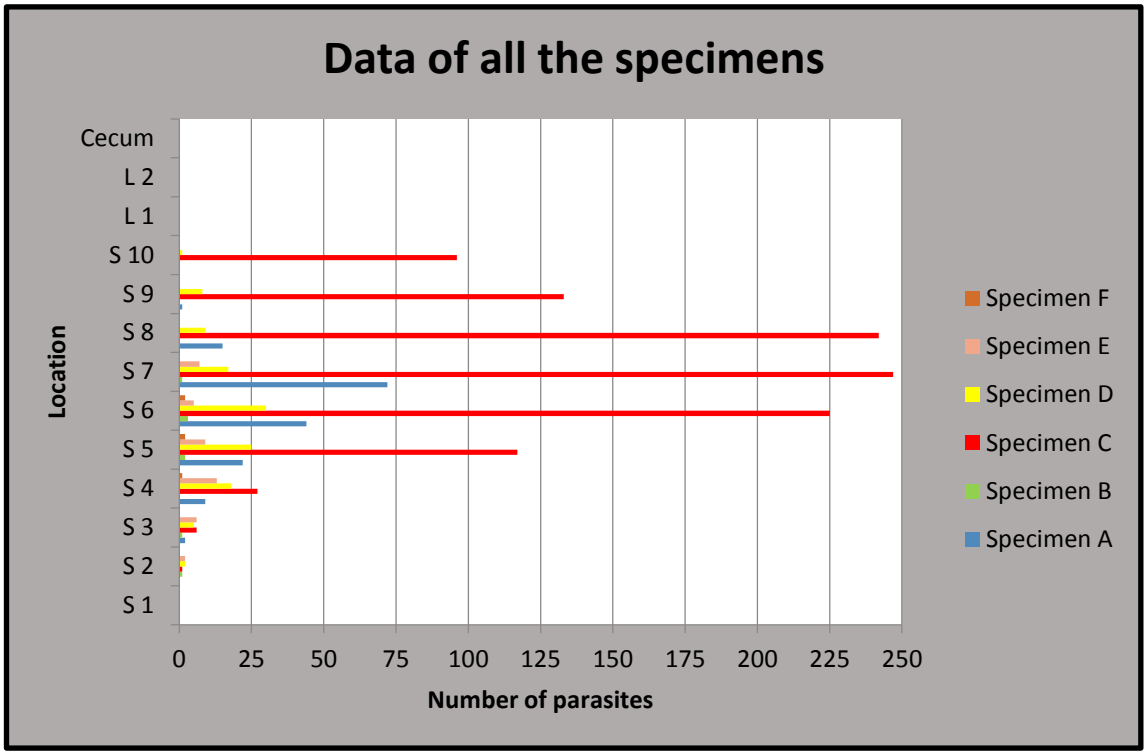


Figure 7 The location of parasites in all of the specimens.

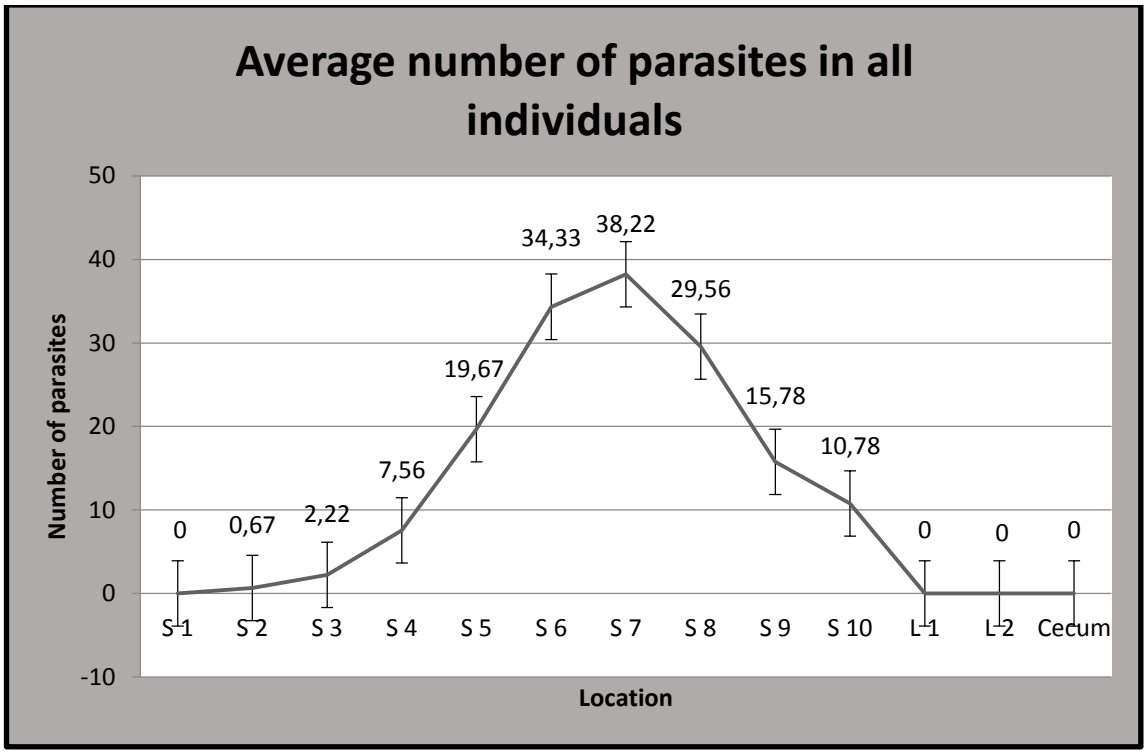


Figure 8 The average number of parasites in all individuals combined, with their standard deviations.

5.2 The correlation between the seal feeding habits and parasites

Corynosoma worms mature after 2–3 weeks in seals. Therefore, it is suggested that the accumulation of parasites over time is not the main reason for the high intensity of infection in some seal individuals, but rather a result of an increased consumption of the paratenic hosts. In other words, an increasing amount of fish eaten by a seal increases its exposure to the parasite. Variations in the number of worms in different seals could then provide a clue about the differences in their feeding habits (Sinisalo et al, 2003; Sinisalo, 2007).

In Lake Saimaa, the pups born from late February to early March are nursed between seven to nine weeks (Hyvärinen et al, 1999). The young pups then learn to catch fish in the shallow waters, where their main prey is perch and roach (Kunnasranta, 2001, Kunnasranta et al. 1999, Auttila et al. 2014). Contrary to the young seals, the adult seals forage mainly in deeper offshore waters (Kunnasranta et al, 2002), which are inhabited by pelagic fish species such as vendace, ruffe and smelt (Jurvelius et al, 1984; Jurvelius & Heikkinen, 1988, Auttila et al. 2014). A higher intensity of *C. magdaleni* infection in the adult seals shows the fish infected with the parasites mainly dwell in offshore waters. The seals take more worms when their diet consists more and more of fish once they reach the age of 6 months (Sinisalo et al, 2003).

5.3 The conservation of the species

This is a limited study, and more research is needed to provide sufficient results.This seal subspecies is critically endangered, and relatively little is known of it. In view of conservation, every study performed on this seal population is extremely important. The study of the parasites in this seal helps determine the general state of health of this animal, and plan further the protection of the subspecies.

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Table of the examined samples

Specimen	ID	Gender	Time of death (mm/yyyy)	Age (y)	Weigth (kg)	Length (cm)	Examination date	Length of small intestine (cm)	Length of large intestine (cm)
A	2529	female	1.7.2011	0	17,5	84	13.5.2014	947	34,5
B	2536	male	x/2011	3	53	122	13.5.2014	940	47,5
C	2541	female	x/2012	1	41	103	14.5.2014	976	22
D	2485	male	1.6.2009	5	32,5	113	15.5.2014	973	?
E	2531	female	x/2011	0	12	91	13.5.2014	500	33,5
F	2527	male	x/2011	0	21	91	16.5.2014	889	30
G	2405	male	x/2006	0	6,1	74	16.5.2014	533	34
H	2361	female	1.3.2004	0	4,3	69	16.5.2014	530,5	26,5
I	2546	female	x/2012	0	8,4	78	15.5.2014	615	17

S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10	L 1	L 2	Cecum	Total parasites	Cause of death
0	0	2	9	22	44	72	15	1	0	0	0	0	**165**	Unknown
0	1	1	0	2	3	1	0	0	0	0	0	0	**8**	Bloodloss
0	1	6	27	117	225	247	242	133	96	0	0	0	**1094**	Asphyxiation
0	2	5	18	25	30	17	9	8	1	Removed			**115**	Unknown
0	2	6	13	9	5	7	0	0	0	0	0	0	**42**	Drowning
0	0	0	1	2	2	0	0	0	0	0	0	0	**5**	Drowning
0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	Unknown
0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	Stillborn
0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	External trauma